A New Pathogenic Micro-Organism of the Conjunctival Sac.

HANFORD McKEE, B.A., M.D.

Assistant Surgeon to the Eye and Ear Department of the Montreal General Hospital; Oculist to the Montreal Maternity Hospital; Assistant Demonstrator in Ophthalmology at McGill University.

From the Pathological Laboratory of the Montreal General Hospital.

[Illustrated.]

Reprinted from the Ophthalmic Record, October, 1907.

A New Pathogenic Micro-Organism of the Conjunctival Sac.

HANFORD McKEE, B.A., M.D.

Assistant Surgeon to the Eye and Ear Department of the Montreal General Hospital; Oculist to the Montreal Maternity Hospital; Assistant Demonstrator in Ophthalmology at McGill University.

From the Pathological Laboratory of the Montreal General Hospital.

[Illustrated.]

A NEW PATHOGENIC MICRO-ORGANISM OF THE CONJUNCTIVAL SAC.*

HANFORD MCKEE, B.A., M.D.

Assistant Surgeon to the Eye and Ear Department of the Montreal General Hospital; Oculist to the Montreal Maternity Hospital; Assistant Demonstrator in Ophthalmology at McGill University.

From the Pathological Laboratory of the Montreal General Hospital.

[Illustrated.]

During the last few years the widening of bacteriological methods in ophthalmology has been extensive. In 1883 Koch, while working in Egypt, examined the conjunctival discharge in some cases of Egyptian ophthalmia. Among the catarrhal forms he found constantly a very small bacillus, similar in size to the bacillus of mouse septicemia. In 1887 Weeks reported having seen epidemics of conjunctivitis in New York, in the conjunctival discharge of which he constantly found a very small bacillus. This was the same micro-organism which Koch had seen in Egypt. The cultivation of this bacillus was extremely difficult. Weeks was at first only able to cultivate it along with the bacillus xerosis, while Koch's attempt at cultivation had been unsuccessful. The organism and the conjunctivitis were called Koch-Weeks. Since described Koch-Weeks conjunctivitis has been studied extensively, the clinical characteristics of the disease and the cultural features of the bacillus established. Its presence has been reported from many different countries, and it is to-day recognized as one of the most contagious and best known diseases of the conjunctival sac.

Neisser in 1879 had described the gonococcus as seen in the pus of gonorrheal affections of the eye, but it was not until after the Koch-Weeks discovery that much activity was shown in studying the bacteriological factors in conjunctivitis.

In 1896 Morax and Axenfeld described a form of conjunctivitis

* Read at the meeting of the American Association of Pathologists and Bacteriologists, Washington, May 9, 1907.

with definite clinical features, which was caused by a diplo-bacillus. This has been called Morax-Axenfeld conjunctivitis. It is to-day one of the most frequently seen diseases of the conjunctiva.

In 1893 Gasparini reported the pneumococcus as a cause of conjunctivitis and keratitis, while still later to the bacillus influenza was attributed a form of conjunctivitis which usually accompanied systemic influenza.

The work thus begun by Koch and Weeks and further pursued by Morax, Axenfeld, Uhthoff and others has borne such fruit that to-day we are able to divide the different forms of conjunctivitis not so much from their clinical characteristics as from the bacteriological findings.

During the last two years we have been doing this bacteriological work at the Montreal General Hospital, to which institution upon April 6, 1907, a mother brought her infant of 9 months and complained that the baby's eyes had been sore and running for five days. The clinical picture was as follows: Both eyes were involved, upon the lashes was much dried secretion. The palpebral conjunctiva was intensely congested, while the bulbar conjunctiva was quite normal. The lids were not red or swollen. From the conjunctival sac there was a profuse muco-purulent discharge freely mixed with tears.

A little of the discharge was smeared well over a glass slide fixed, and stained by Gram's method, using as a counter-stain a weak solution of safranin. Upon examining the slide I saw here and there tiny Gram negative bacilli which seemed too short and thick for the Koch-Weeks bacillus and too thick for the bacillus influenza. This same bacillus I had seen before, but in the hurry of a large outdoor clinic I had not pursued it further, contenting myself with the thought that it was the bacillus influenza.

Tubes of blood serum, plain agar, bouillon and hemoglobin agar were then inoculated and put in the incubator. After twenty-four hours a growth was seen upon the hemoglobin agar. The other tubes were negative. Smears showed this same small Gram negative bacillus with staphylococci. The bacillus, after a few days, was obtained in pure culture. A conjunctival sac was now inoculated, a conjunctivitis set up, and from the conjunctival discharge the same small bacillus was obtained.

Within a month nine such cases have presented themselves at the outdoor department. They have all been infants and all from among the Jewish colony here. They have all shown definite characteristic clinical features and from each case this same bacillus has been isolated.

SMEAR PREPARATIONS.

In making a smear a loopful of the conjunctival discharge should be teased well over a glass slide, fixed, and stained preferably by Gram's stain, using as a counter-stain a 5 per cent. watery solution of safranin. Gram's stain is especially useful in conjunctival work, eliminating immediately the frequently present Gram positive bacilli. Upon examining the smear one finds here and there tiny bacilli. They are short and thick, their rounded ends giving the appearance of elongated cocci. They may be found in groups, but seem to lie preferably singly. They will be found both within and without the leucocytes, generally without.

From culture the bacillus seems to be slightly larger and tends to appear as diplo-bacilli. It is short and thick, with very pointed ends, is 0.5-2.0 microns long by 0.3-0.4 microns wide. The bacillus shows uniform staining, has no capsule, is non-motile and does not form spores. It is decolorized by Gram's stain, but stains well with weak solutions of carbol fuchsin or methylene blue.

CULTURAL FEATURES.

The tubes of hemoglobin-agar inoculated with some of the conjunctival discharge gave a pretty and characteristic result, which was constant in all cases. After twenty-four hours in the incubator one sees over the surface numerous separate colonies of the staphylococcus albus. Around each of these separate colonies radiating out from them as centers are seen very fine colorless shiny rounded colonies. These colonies are exceedingly fine, seem to keep separate and extend over the whole surface. The original cultures from all the cases were as the one described and showed how well this bacillus grows in commensal relationship with the staphyloeoccus.

From the original tube the bacillus was obtained in pure culture by surface seeding a plate of hemoglobin-agar. In trying to obtain it in pure culture by transferring from tube to tube the growths were always contaminated by the staphyloeoccus. Over a plate of

hemoglobin-agar, however, by smearing a loop inoculated from one of the small colonies as far from the albus as possible the bacillus in pure culture was readily obtained.

The growth of the bacillus on hemoglobin-agar is characteristic: After twenty-two to twenty-four hours in the incubator the slant will be seen covered with a mass of tiny colonies. They remain separate and distinct, are colorless, and, as will be seen from the tubes, the colonies are no larger than the sharp end of a pin. Indeed, the growth, though profuse, is at times exceedingly difficult to recognize. It is seen much better by artificial than by daylight, especially with the proper reflection of light. The growth of the bacillus is very easily affected even upon hemoglobin-agar, upon which it grows best. Upon freshly-made hemoglobin-agar it will not grow in less than forty-eight hours. The reaction of the media, too, is of the greatest importance. Hemoglobin-agar, neutral, 1.0 alkaline, .65 acid, 1.0 acid and 1.75 acid to phenolphthalein was made up. Upon alkaline media it would not grow. Upon the neutral and .65 it was with great difficulty transferable. The most satisfactory reaction and the one which has been latterly used is the 1.0 and 1.75 acid.

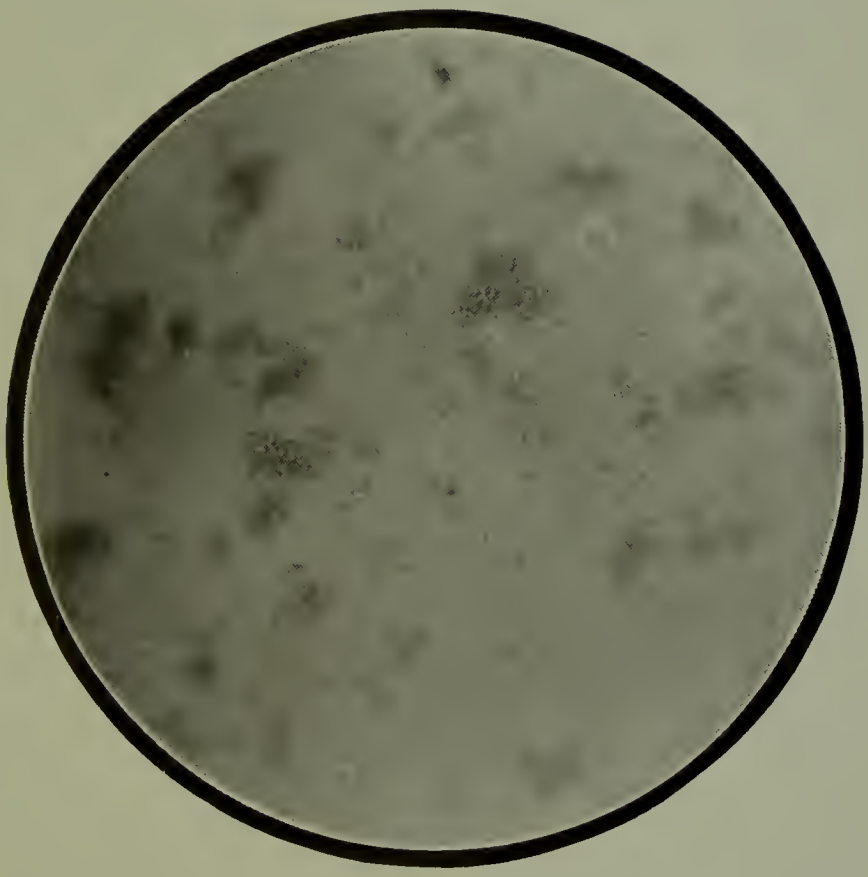
The cultivation of this bacillus on media, upon which it seemed to thrive best, has been at times unsatisfactory and extremely difficult. Upon the same batch of hemoglobin-agar sometimes it will take forty-eight hours before there is any sign of a growth. At other times cultivation is comparatively easy.

Upon glycerin agar, after twenty-four hours in the incubator, a growth is seen. The surface of the agar is covered with these same tiny pin-point colorless colonies. They are here hardly perceptible, but a smear from the surface will show their presence in quantity.

Upon hydrocele agar the growth is exceedingly fine and is only perceptible with a proper reflection of light. From behind no growth will be seen, but smears from the surface will show the presence of the organism. . Upon plain agar the appearance of the growth is similar to that upon hydrocele agar. The growth upon plain agar is by no means constant.

Upon blood serum the results have been negative.

In bouillon there has never been any growth: indeed, even in



Bacillus from cases of acute catarrhal conjunctivitis. Oil immersion 1/12 O.c. No. 3.

the water of condensation from a slant where there is a profuse growth the bacilli are very few in number.

To tubes of litmus agar were added dextrose, dextrin, maltose lactose. saccharose, gelatose, innulin and mannit. The growths here were sparse. Over the surface of the agar were seen tiny colorless pin-point colonies.

To similar litmus agar tubes to which sugars had already been added were added a few drops of blood. The growth here was profuse, with no change in the reaction. The bacillus, too, has been cultivated upon dorset egg medium. The appearance of the growth is as if the surface of the medium had been slightly roughened with a swab stick.

To sum up its cultural characteristics, this bacillus grows only at the body temperature and is aërobic. It grows best upon hemoglobin-agar of 1.0 and 1.75 acid reaction. The growth upon the hemoglobin-agar is very easily affected and the cultivation of the bacillus is at times difficult. In commensal relationship with staphylococcus albus the bacillus grows well.

It grows on dorset egg medium, glycerin and hydrocele agar and has been cultivated upon plain agar. It does not grow in bouillon, any liquid medium or upon blood serum. It does not produce gas, does not ferment any sugars and has no chromogenic characters.

VIABILITY.

The cultures on hemoglobin-agar remain viable from two or three weeks, provided there is sufficient water of condensation in the tubes. One culture inoculated on April 11 was transferable April 25 but not on May 3. It had not been kept in the cold nor in any way taken especial care of.

PATHOGENICITY.

For the human conjunctival sac this bacillus is pathogenic. A conjunctival sac which was found to be free from micro-organisms was flushed well out with warm water. Fifteen minutes later the sac was inoculated with a loopful of a twenty-four hour growth. Twelve hours later there was a feeling of "something in the eye," and six hours later the eye showed a marked catarrhal conjunctivitis. The palpebral conjunctiva of the lower lid was especially involved. The

posterior conjunctival vessels were somewhat dilated, otherwise the bulbar conjunctiva was not involved. From the muco-purulent discharge, which was here, too, freely mixed with tears, the organism was obtained.

With the washing from a slant on hemoglobin-agar an ordinary house mouse was inoculated intraperitoneally; eighteen hours later the mouse was unable to move and six hours later was dead. At autopsy the peritoneum was found injected and filled with a gelatinous sticky exudate. Smears showed the pus cells filled with the bacilli and little or no fibrin. Inoculations from the peritoneum on hemoglobin-agar gave pure growths of the bacillus.

This tube was washed down and inoculated intraperitoneally into a white mouse. Twenty hours later the mouse was dead. Postmortem was found marked peritonitis of the gelatinous type similar to Case 1. From the peritoneum here was obtained the bacillus in pure culture. A different strain of the bacillus on hemoglobin-agar was now taken and the wash similarly injected into a white mouse. Sixteen hours later the mouse seemed very ill and was unable to stand up or move, and four hours later was dead. From the peritoneum, which showed a marked peritonitis similar to the previous cases, two tubes of hemoglobin-agar were inoculated, which twenty-four hours later gave pure growths of the bacillus. Hemoglobin agar was also inoculated with the heart's blood, which twenty-four hours later gave a pure growth of this same bacillus. Two house mice were inoculated with two different strains; twenty-four hours later they were both dead. Postmortem was found the marked gelatinous peritonitis, from which pure cultures were obtained, also pure cultures from the heart's blood. In one case two tiny loopsful from the heart's blood gave a growth covering the entire surface of the agar. To check these experiments on April 29 last four mice, two white and two house mice, were inoculated intraperitoneally with four different strains of the bacillus. The inoculations were done at 4 p. m. on April 29. At noon the following day one white mouse and one brown one were dead. The other two were unable to move or stand. Postmortem was found the peritoneum in all cases involved as before. From the peritoneum and from the heart's blood in each case were obtained pure growths.

This micro-organism then, a cause of conjunctivitis, needs to be differentiated from the different known bacteriological causes of conjunctivitis. Differentiation from Morax-Axenfeld's diplobacillus. Petit's diplo-bacillus, Friedländer's bacillus and bacillus coli and bacillus subtilis may be dealt with in a few words. It is far too different morphologically and culturally to necessitate going into minutiæ here. The pathogenic micro-organisms of the conjunctival sac, which it must be differentiated from, however, are the Koch-Weeks bacillus and the different influenza bacilli, namely, the influenza bacillus of Pfeiffer, the so-called influenza baeillus of conjunctivitis of Müller and the pseudo influenza bacillus of Zur Nedden.

1. *Clinical Considerations.*—While the different bacteriological factors causing conjunctivitis do not always, yet in the majority of cases these organisms do give rise to well-marked eharacteristic clinical pictures. In Koch-Weeks conjunctivitis one finds reddened and edematous lids, intense injection of the bulbar conconjunctiva, with profuse mueo-purulent discharge. In influenza conjunctivitis there is a well-marked involvement of the bulbar conjunctiva and signs of influenza elsewhere. The bacillus influenza has been found in the normal conjunctival sac and in odd cases of conjunctivitis where there were no general signs of influenza, but in the majority of cases influenza conjunctivitis accompanies systemic influenza. Clinically, Koch-Weeks, influenza, and the form of conjunctivitis here described are very different.

2. *Morphology.*—Differentiation by morphology is unsatisfaetory and here unnecessary. While the Koch-Weeks bacillus may be differentiated from the bacillus influenza, I am ready to admit that morphologically they are strikingly similar.

3. On media these organisms differ widely. The Koch-Weeks bacillus does not grow well on hemoglobin agar as here used, it grows best on hydrocele or ascitic agar. When growing even with the bacillus xerosis it is hardly perceptible and dies out very quickly in forty-eight hours or a little longer.

The bacillus influenza in the presence of hemoglobin after twenty-four hours shows a profuse raised growth of round whitish

colonies. The growth is easily seen, the colonies being the size of an ordinary pin head. Older colonies show some change in color.

The bacillus here described on hemoglobin agar gives a very characteristic appearance; over the surface of the agar, with a proper reflection of light, is seen a mass of tiny pin-point colorless colonies. The growth is not easily seen. This organism has also been cultivated on media free from hemoglobin.

4. *Pathogenicity.*—The Koch-Weeks bacillus, while pathogenic for the human conjunctival sac, is not pathogenic for animals. The bacillus influenza is slightly pathogenic for some animals. Pfeiffer found by injecting intravenously into rabbits the bacillus influenza a characteristic effect was produced. He attributed these results to toxic products present in the cultures, and in none of his experiments was he ever able to obtain effects resembling septicemic infection. Cantani by injecting the bacillus influenza into the anterior portion of the brain of rabbits succeeded in producing an acute encephalitis. The bacilli, however, were never found in the blood or other organs.

This bacillus is pathogenic for mice. In every case where injected intraperitoneally a marked peritonitis was set up and a septicemia, as evidenced by pure cultures of the bacillus, being obtained from the heart's blood.

This organism then is similar to the different influenza bacilli in morphology and in its growing well in commensal relationship with the staphylococcus.

It is not bacillus influenza for the following reasons:

(1) The form of conjunctivitis set up differs from influenza conjunctivitis.

(2) This bacillus on hemoglobin agar differs widely from bacillus influenza. On this media, under most favorable conditions and with the media at a most suitable reaction, the growth is hardly perceptible and differs from the bacillus influenza on the same media in size, color and appearance.

(3) The bacillus has been cultivated on media free from hemoglobin.

(4) It is viable for a much longer period than the bacillus influenza.

(5) It is pathogenic for mice causing a septicemia.

CONCLUSIONS.

We have, then, here to deal with a new form of conjunctivitis set up by a new etiological factor. That factor resembles somewhat the baeillus of influenza, but differs from it widely on media, viability and pathogenicity. From the examination of 500 cases of conjunctivitis during the last year and a half this series of cases, occurring within a month, seems to be different from previously described types. Nine cases I recognize is, indeed, a small number to draw conclusions from, but the characteristic clinical features of these cases caused by an organism not as yet described as a pathological factor in conjunctivitis leads me to the conclusion that we have here to deal with a new form of conjunctivitis set up by a new organism of the conjunctival sac.

Until the clinical features from which the bacillus should take its name are better understood I shall call it baeillus McKee.

I take great pleasure in expressing my thanks to Dr. C. W. Duval, the director of the laboratory, for much assistance while doing this work.